

Research Article

A rapid method for determination of commercial β -carotene in RBD palm olein by Fourier transform infrared spectroscopy

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Abstract

A rapid method for the determination of commercial β -carotene in refined bleached and deodorized (RBD) palm olein using Fourier transform infrared (FTIR) spectroscopy was developed. The fifty RBD palm olein samples spiked by a known amount of commercial (30%) β -carotene to produce a wide range of concentrations up to 2000 ppm were used. Samples were separated into two groups for the calibration and validation models. The partial least squares (PLS) calibration models for predicting β -carotene was developed by using the FTIR spectral region at 980-915 cm^{-1} which is associated with trans double bond CH absorption. The accuracy of the method was comparable to that of the HPLC method with a coefficient of determination (R^2) and standard error of calibration (SEC) for commercial β -carotene 0.9934 and 52.29, respectively. The FTIR method developed was shown to be efficient, accurate and suitable for routine quality control analysis for the food industry with results obtainable in about 2.5 min.

Keywords: FTIR, food analysis, CH absorption, SEC, cooking oil, Malaysia, Thailand

Introduction

Palm olein is the liquid obtained by fractionation of palm oil following crystallization. The characteristics of palm olein differ from those of palm oil and it is used worldwide as a cooking oil. It is also highly sought after as it blends well with other cooking oils. Palm olein exhibits good resistance to oxidation and breakdown when frying and provides longer shelf life of food fried with olein. RBD palm olein is olein which has been refined, bleached and deodorized. Carotene occurring naturally in palm oil or olein is lost during refining, so many companies are now adding carotene prior to product sale. Red palm olein gets its colour from the high carotene content and this form has currently become commercially available.

Carotene has been reported to play a role as an antioxidant in lipid phase by trapping free radicals or by quenching singlet oxygen. By far the most common carotene found in plant tissue is β -carotene and this has been intensively studied [1]. It is recognized that β -carotene is an effective quencher of singlet oxygen, hence acts as an antioxidant and prevent the damage normally caused by them [2]. The order of their free radical scavenging functional groups on the rings were also found to affect radical scavenging capacity. β -carotene has been shown to act as a pro-oxidant during lipid oxidation, both in the light and in the dark at higher concentrations [3]. β -carotene content is normally determined by using high performance liquid chromatography (HPLC) [4, 5].

Fourier transform infrared (FTIR) spectroscopy is rapidly becoming the method of choice for data calibration, particularly where quantification is the main aim [6]. FTIR spectroscopy is a powerful instrument for both qualitative and quantitative analysis in edible oils owing to the substantial functional group information contained within the IR spectrum [7]. It has been used previously in the non-chemical determination of quality parameters of edible oil with various calibrations ranging from the simple Beer's law to sophisticated multivariate analysis [8]. Furthermore, the FTIR method has been shown to provide higher accuracy and a significant reduction in total analysis time (2.5 min/sample) as well as being amenable to automation [9]. FTIR has been applied for analytical studies such as measurement of gossypol in cottonseed oil [10], α -tocopherol in RBD palm olein [11], propyl gallate in RBD palm olein [12] and butylated hydroxytoluene in RBD palm olein and RBD palm oil [13]. The aim of this research work was to assess the suitability of FTIR spectroscopy to measure commercial β -carotene in RBD palm olein.

Research Methodology

Sample and sample preparation

RBD palm olein without added antioxidants was purchased from a local palm oil refinery in Malaysia. Commercial grade β -carotene (30 % in corn oil) was purchased from Roche, Germany. All reagents were of analytical grade. Fifty RBD palm olein samples were spiked by known amounts of commercial β -carotene to concentrations up to 2000 mg/kg (ppm) of β -carotene content in oil samples. Each set of samples were separated into two groups for the calibration and validation models.

Instrumental analysis

HPLC analysis

HPLC was used to determine β -carotene in the spiked samples of RBD palm olein. The HPLC system used was equipped with UV visible detector with the wavelength 446 nm (SPD-10 AV, Shimadzu, Tokyo, Japan), HPLC pump (LC-10AT), a column oven (CTO-10A), TSK gel ODS-80TS column 4.6 mm ID x 25 cm. The mobile phase was acetonitrile/dichloromethane (80:20 v/v) with a flow rate of 1 mL/min.

FTIR analysis

Perkin-Elmer FTIR Spectrum BX, (Perkin-Elmer Corporation, Norwalk, CT, USA), with a room temperature deuterated triglycine sulphate (DTGS) detector. The spectrometer was connected to a computer using Perkin-Elmer Spectrum Windows software to manipulate the spectra. The instrument was maintained with two automatic dehumidifiers to minimize interference from water vapour.

Melted drops of each sample were placed between the sodium chloride (NaCl) windows, and the transmission path was fixed at 50 μ m by adjusting the polytetrafluoroethylene (PTFE) spacer. The cell was then placed in the cell holder and the sample scanned. After each sample scanning, the NaCl windows of the transmission cell were rinsed three times with acetone and then dried with soft tissue for the next sample.

Statistical multivariate analysis

All experiments and measurements were done in duplicate. The relationships between each FTIR spectrum parameter and data from HPLC analysis were determined using the software program Nicolet Turbo Quant IR-Calibration and Prediction Package, Version 1.1 (Nicolet Instrument Co., Madison, WI).

Calibration development and validation

Fifty samples were used, with 38 samples in the calibration sets and the remaining 12 samples in the prediction set. PLS regressions were used to analyze the concentration data from the samples. Only the concentrations need to be known.

In PLS, the HPLC data and spectral data were correlated and correlation coefficients (R^2) were taken as estimates of the factor scores, which then were used as regressors to model both the spectral and chemical data.

Results and Discussion

Development of calibration and validation models

HPLC analysis was performed to determine the initial carotene content in the oil. The result showed that no carotene was found in fresh RBD palm olein from a local palm oil refinery. These results are in close agreement with May [14], who reported that all carotenes are destroyed in the conventional physical refining process. The fifty RBD palm olein samples were spiked by commercial β -carotene for the FTIR calibration and HPLC analysis and the data were used to estimate the calibration range up to 2000 ppm.

Spectral analysis

Figure 1 shows the spectrum of β -carotene. The β -carotene spectrum exhibited peaks at 2922 cm^{-1} and 2862 cm^{-1} for asymmetric and symmetric stretching vibrations of the CH_2 and CH_3 [15], 1445 cm^{-1} for CH_2 scissoring, 1360 cm^{-1} for splitting due to dimethyl group, 1033 cm^{-1} for in plane $-\text{CH}-$ [16] and 962 cm^{-1} for trans conjugated alkene $-\text{CH}=\text{CH}-$ out-of-plane deformation mode [17]. Figure 2 illustrates 5 overlaid FTIR absorbance spectra of samples containing commercial β -carotene in RBD palm olein in different concentrations. The absorption changes at 980-930 cm^{-1} as trans $=\text{CH}$ stretching band with the changes in concentration of β -carotene (A) 0 ppm, (B) 500 ppm, (C) 1000 ppm, (D) 1500 ppm, (E) 2000 ppm. Eight spectral regions, (3050-2775 cm^{-1} , 1690-1578 cm^{-1} , 1490-1406 cm^{-1} , 1406-1296 cm^{-1} , 1222-980 cm^{-1} , 980-915 cm^{-1} , 1490-1296 and 1222-915, respectively) were used to build the calibration models for commercial β -carotene determination.

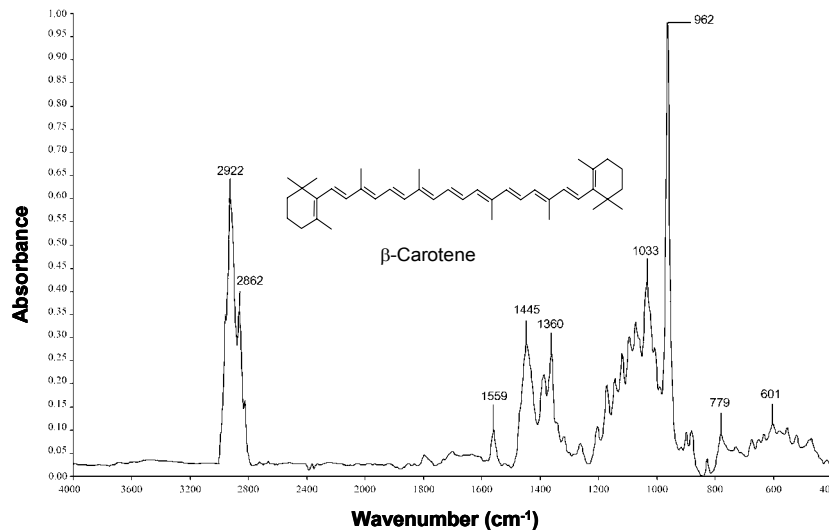


Figure 1. FTIR spectra of β -carotene.

The calibration standards were designed to obtain data for the PLS regression. Table 1 shows the results obtained from the PLS calibration in terms of the coefficient of determination (R^2), the standard errors of calibration (SEC) and the standard errors of prediction (SEP) of commercial β -carotene used in choosing the best regions for determining β -carotene in RBD palm olein. It was found that FTIR spectral regions 980-915 cm^{-1} (trans $=\text{CH}$ region) were the best for commercial β -carotene with lowest SEC (52.29) and SEP (53.96) and highest R^2 (0.9934).

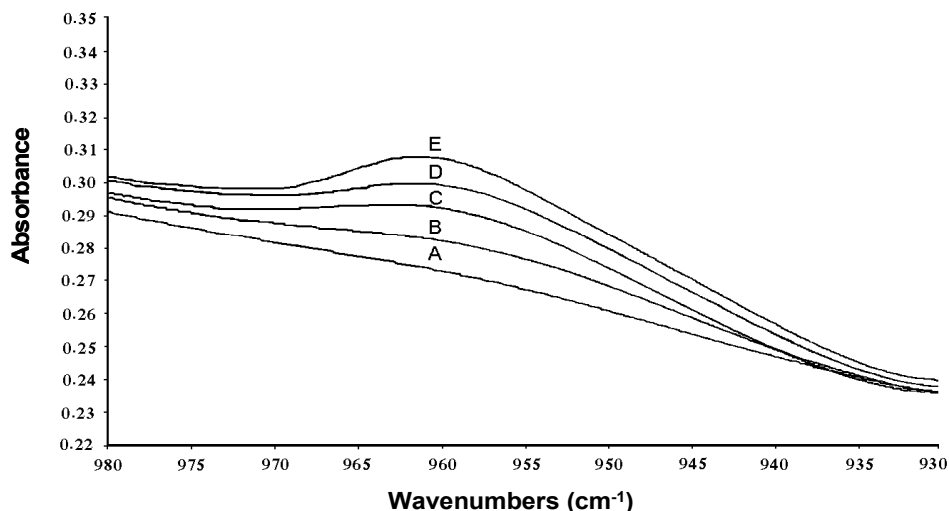


Figure 2. Absorption changes at 980-930 cm^{-1} with different concentrations of β - carotene (A) 0 ppm, (B) 500 ppm, (C) 1000 ppm, (D) 1500 ppm, (E) 2000 ppm.

Table 1. Effect of different wavelength regions in developing a calibration model for determining β -carotene content in RBD palm olein^a

Region No.	Wavelength Region (cm^{-1})	Commercial β -carotene		
		R ²	SEC	SEP
1	3050-2775	0.7192	330.2	201.8
2	1690-1578	0.4115	455.2	104.0
3	1490-1406	0.6738	370.8	222.8
4	1406-1296	0.7350	321.7	190.3
5	1222-980	0.6816	353.8	209.9
6	980-915	0.9934	52.29	53.96
3+4	1490-1296	0.6891	357.0	227.30
5+6	1222-915	0.6912	348.2	216.8

^aR², coefficient of determination; SEC, standard errors of calibration; SEP, standard errors of prediction.

Figure 3 shows the correlation plot of commercial β -carotene by using the actual data from HPLC analysis that gave the coefficient of determination (R^2) of 0.9934 and the equation of $Y = 0.9934x + 6.7386$. Figure 4 shows a validation plot of commercial β -carotene in RBD palm olein with R^2 of 0.9780 that tested the calibration validity. In general, for n components to be analyzed, at least $(2n+2)$ standards are needed for the calibration [18].

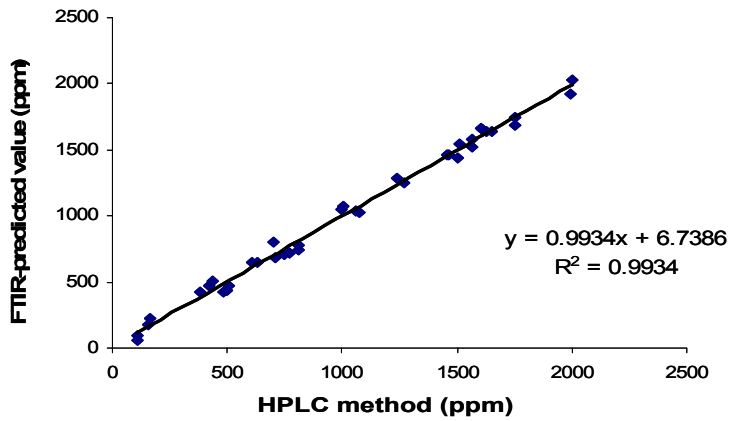


Figure 3. Calibration plot of the actual data from HPLC analysis of commercial β -carotene in RBD palm olein from 38 samples versus the PLS FTIR predicted values.

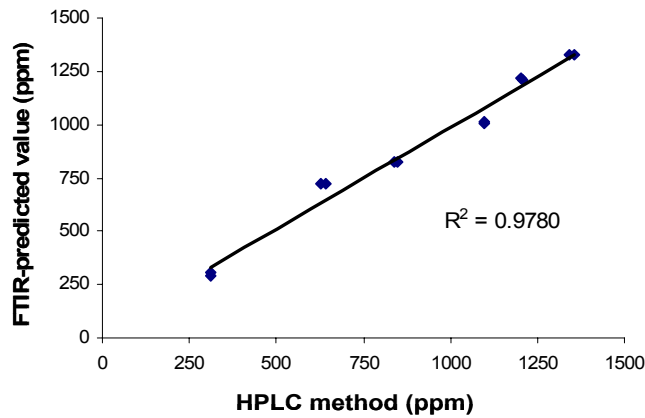


Figure 4. Validation plot of the HPLC analysis data from 12 samples versus the PLS FTIR predicted values.

Table 2. Calibration and validation statistics for commercial β -carotene in RBD palm olein determined by HPLC method and FTIR spectroscopic method using PLS of wavelength regions 980-915 (cm^{-1})^a

Data Set		HPLC method			FTIR method		
		Mean	SD	CV (%)	Mean	SD	CV (%)
Commercial β -carotene	Calibration	1022.53	16.88	1.65	1020.03	15.62	1.53
	Validation	907.20	8.51	0.94	898.13	9.98	1.11

HPLC, high performance liquid chromatography, FTIR, Fourier transform infrared spectroscopy; SD, standard deviation; CV, coefficient of variation; all the data were the means from two replicates.

Table 2 shows the mean, standard deviation (SD) for reproducibility between duplicates and the coefficient of variation (CV) of commercial β -carotene content in RBD palm olein sample sets which were obtained from both the calibration and validation models. The values of mean and SD obtained from calibration and validation sets of commercial β -carotene by HPLC analysis were 1022.53, 16.88 and 907.20, 8.51, respectively, while for the FTIR were 1020.03, 15.62 and 898.13, 9.98, respectively. The CV obtained using the PLS statistical method to predict FTIR results from the HPLC method of commercial β -carotene sample sets for calibration sets were 1.53%, respectively, while for validation sets were 1.11 %, respectively. A CV less than 20% is acceptable for most analytical purposes as reported by Che Man and Mirghani [19].

Table 3. Calibration statistics of commercial β -carotene in RBD palm olein from data obtained by HPLC method and FTIR spectroscopy^a

Statistic	Commercial β -carotene	
	HPLC	FTIR
MD _r	2.26	3.43
SDD _r	1.94	1.26
Min. value	106.81	89.52
Max value	2000.48	2032.00
MD _a	2.12	
SDD _a	0.67	

^a MD, mean difference; SDD, standard deviation of difference; r, repeatability; a, accuracy; FTIR, Fourier transform infrared spectroscopy; all the data were the means from two replicates.

The results of statistical analysis (Table 3) show the mean difference for repeatability (MD_r) were very low for HPLC and FTIR methods in commercial β -carotene sample sets, implying that there was little difference between them in the two series of analyses. The standard deviation of difference for repeatability (SDD_r), which measures variability around the MD, was also very low in both FTIR and HPLC methods. The result in terms of the mean difference for accuracy

MDa and the standard deviation of difference for accuracy (SDDa) indicated that β -carotene in RBD palm olein could be measured with good accuracy by FTIR spectroscopy.

Conclusion

Satisfactory results for predictions of commercial β -carotene in RBD palm olein has been shown by using FTIR spectroscopy in our research. The method was based on the sodium chloride (NaCl) windows. The partial least squares (PLS) calibration models were developed by using the FTIR spectra which gave the best spectral region at 980-915 cm^{-1} that is associated with trans double bond CH absorption that gives the coefficient of determination (R^2) and standard error of calibration (SEC) for commercial β -carotene of 0.9934 and 52.29, respectively. The complete FTIR analysis to determine β -carotene in RBD palm olein can be performed in less than 2.5 minutes per sample. Further advantage of the FTIR method is that it is environmentally friendly, as no chemicals are needed for the analysis.

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